Continued from March 1982

From

SINNESORGANE IM PFLANZENREICH

by Gottlieb Haberlandt

Insectivores: *Dionaea muscipula*

Translated by Carla R. Powell
Departments of Chemistry and Foreign Language
Lebanon Valley College, Annville, PA 17003

Upon treatment with zinc chloride-iodine solution[3], these underlying cell wall layers exhibit only a light yellowish-grey coloration, while the epidermal outer walls of the leaf blades up to the cuticle immediately turn an even shade of blue. There can scarcely be a doubt that, just as in the case of *Aldrovanda*, the differing chemical behavioral of the outer walls of the hinge cells correlates with their flexibility and elasticity.

The radial longitudinal walls (side walls) of the hinge cells are considerably thickened. This is easily observed on crosswise microtome sections (Plate VII, Fig 2) and tangential longitudinal sections through the hinge (Fig. 5). On tangential longitudinal sections one also sees that the upper and lower peripheral portions of the longitudinal walls are thinner and, as a result, the cell lumina are enlarged at these points. The longitudinal walls turn blue and swell greatly when treated with zinc chloride-iodine[3]. Even without further treatment, after the protoplasts have been fixed with chrome-osmium acetic acid[8], a delicate cross-striation of the somewhat swollen longitudinal walls is observed, resulting from the presence of more numerous plasmodesmata between the adjoining hinge cells (Plate VII, Fig 3)[9]. The sloping lateral walls, which border on the hinge cells above and below, are just as thin as the radial walls of the adjacent epidermal cells, corresponding to them.

The inner walls of the hinge cells are again quite thick (Plate VII, Fig. 1); but the thickening does not usually extend all the way to the upper and lower transverse edge of the inner wall. These walls also are traversed by plasmodesmata[9], such that the protoplasts of the hinge- or sensory cells are connected not only with one another, but also with the protoplasts of the central cell bundle.

This cell bundle, surrounded by 15-16 hinge cells in the form of a ring, consists of longitudinally stretched cells with perpendicular, or only slightly slanted, transverse walls (Plate VI, Fig 10). The bundle is, as a rule, two cell layers high, and on median lengthwise sections, three cell layers wide. In the case of an exceptionally weakly constructed bristle, I found a single longitudinally stretched cell in place of the entire bundle. The content of these cells includes a living plasmatic body with an ellipsoidal nucleus. The nature of their walls is peculiar. They are more or less thickened, and, with relatively weak magnification, appear as though they are covered with rounded pits. (This corrects the mistaken description of these cell walls in the first edition of this book, p. 113.) Renewed investigation, however, explained this appearance. Many strongly refractive granules, or sinuate platelets, are embedded in the middle lamellae in a single layer. To judge by their reactions, they consist of a cutose substance[10]. On thin microtome sections (5-10 μm thick) one sees very clearly, with sufficiently strong magnification, that the rows of granules are covered on both sides by smooth cellulose lamellae, which correspond to the layers of secondary thickening (Plate VII, Figs 1, 2). Such cutin granules also occur in the partition walls between the hinge cells and the central cells. It is worth noting that, except for the longitudinal walls, only the transverse walls inside the hinge cells exhibit this construction. The upper- and lowermost transverse walls, through which the cell bundle borders on the adjacent tissue, consist of relatively pure cellulose and have no granules. On the whole, those characteristic wall properties are not so much determined by the cell boundaries, as much more by the upper and lower boundaries of the hinge. Here, the height of the hinge is equal to the height (or length) of the inner walls of the

epidermal hinge cells. The walls of the cells existing within these boundaries and the cell parts exhibit the qualities just described. Therefore, it may be concluded that these characteristic wall properties are connected with the function of the hinge. The importance of the deposition of numerous cutose granules in the cellulose wall is a complete mystery.

In the first edition of this book, I considered the central cell bundle in question to be the mechanical tissue of the entire hinge, and compared it, as far as its function is concerned, with the central body of leaf pulvini. This conception was based on the incorrect assumption that the radial longitudinal walls of the hinge cells were thin and delicate, such that the presence of a central mechanical cell bundle would be conceivable. Now, however, the laminar hinge cells in general appear to be so thick-walled that a special mechanical cell bundle in the middle of the hinge probably is superfluous. Since there are plasmodesmata between the hinge cells and the central cell bundle, the most important function of the bundle must be to transmit to the pedestal, or lamina, the state of excitation caused by deformation of the hinge cells[11].

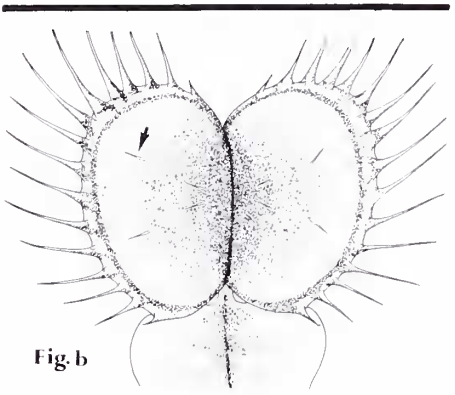

Fig. b

Fig. b. A large leaf of *Dionaea* in its spring form. An arrow points to one of the bristles (trigger hairs) on the surface of the leaf. This particular leaf has eight bristles as many larger leaves do. Leaves with six bristles would have one bristle, in place of the centrally located pairs of bristles, located on each side of the blade exactly between the pairs of bristles nearest the midvein. Haberlandt's Fig. 10 in Plate VI is a longitudinal section through the base of one of these bristles.

4. The undermost part of the tactile bristle is formed from a cylindrical pedestal which is approximately as high as it is wide. It consists of thin-walled, plasma-rich parenchymatous cells, which are occasionally faintly collenchymatous, and are surrounded by an epidermis having rather elongated cells. It is worth noting that only the innermost layers of the moderately thickened outer walls of these epidermal cells are stained blue by zinc chloride-iodine, while the outer layers take on the same yellowish-gray color as the outer walls of the hinge cells, without being cuticularized. This indicates that when the bristle is more severely bent, both the actual hinge and the pedestal are slightly bent. In agreement with this, the pedestal is not broadened at its base. Indeed, it is inclined to be thinner here than it is further above. (See Haberlandt, 1896, Fig. 207; Goebel's illustration is incorrect in this issue.) By more severe bending of a bristle under a microscope, one can readily observe that, in fact, the pedestal is also bent (Haberlandt, 1896, Fig. 208). And indeed this bending is provided for in the structure of the pedestal. When the bristle is severely bent, as it is particularly after the leaf halves close, its function evidently is to prevent a too great, possibly permanently damaging deformation of the hinge. However, with weaker impulses this is probably the only place bending will occur. The strongest bending will undoubtedly occur in the hinge zone characterized by the ring-shaped constriction.

I must now discuss briefly the nature of the deformations of the protoplasts of the hinge cells caused by their bending.

On observation of a median longitudinal section through the bristle, one sees that the hinge furrow is actually none other than a crease in the thickened outer walls (Plate VI, Fig. 10 and Plate II, Fig. 1). Each hinge cell, or sensory cell, has in its very thick outer wall a much thinner cross-striation. When the hinge is bent, the convex side is greatly stretched, while the concave side forms a fold at this point (Haberlandt, 1896, p. 208). When the convex side is stretched, the portion of the plasma membrane adjoining the thin wall striation is subjected to extreme stretching, and this deformation of the protoplast, or plasma membrane, is evidently greater here than at any other place on the cell deformed by the bending. On the concave side the nature of the mech-

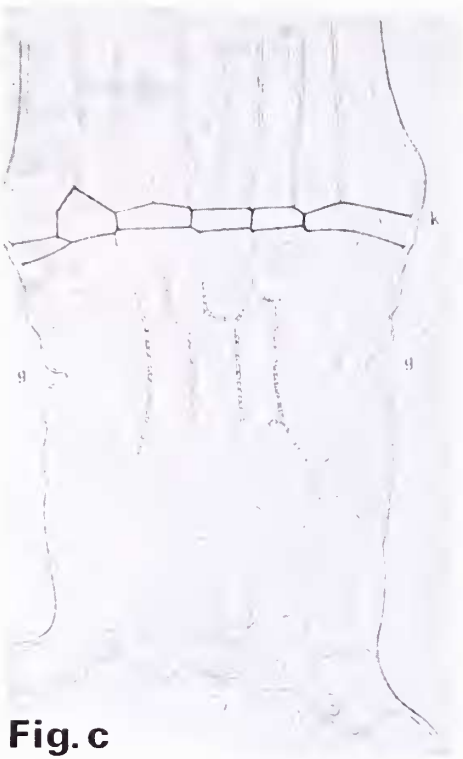

Fig. c

Fig. c. Plate IV fig. 10 from the second edition of *Sinnsorgane*. Plate VI is the same in both the first and second edition of the book except for this drawing (c.f. Plate VI Fig. 10). Note that Haberlandt changed his drawing in the second edition to indicate that all walls of the cells in the middle layer (k) are suberized instead of just the uppermost walls. Because of the much lower quality of printing of the plates in the second edition photographs of Plate VI are taken from the first edition.

anical demands placed on the plasma membrane cannot be so precisely defined. In the case of a weaker bending, it may have been subjected to a longitudinal pressure. On the other hand, with a strong bending whereby the thin portion of the outer wall folds inward, a longitudinal stretching of the plasma membrane combined with radial compression probably occurs. In any case, however, the important difference in the thickness of the outer walls of the hinge cells serves to localize the deformation on a very specific part of the plasma membrane. That the stretched plasma membrane is especially sensitive at these places, that is, under the only slightly thickened hinge striations of the outer walls, is an obvious surmise[12].

It is possible to explain more clearly the workings of the hinge furrow when the bristle is bent, if we calculate and compare with one another the bending moments of two hollow cylinders which we imagine to represent the outer walls of the hinge cells at their thickest and thinnest points. The formula for the bending moment[13] of a hollow cylinder in which the contents are neglected is: $F(r_1^2 + r_2^2)/4$. In this formula F represents the cross-sectional area, and r_1 and r_2 the radii of the hollow cylinder differing by the thickness of the wall (Schwendener, 1874, p. 23). I determined the following values for F, r_1, and r_2 on the basis of measurements of a drawing of the median longitudinal section through the hinge, carried out by means of a [camera lucida]. In the case of a hollow cylinder whose wall is formed from the thickest portion of the outer walls of the hinge cells, $F = 405$ mm^2, $r_1 = 23$ mm, and $r_2 = 20$ mm. The size of the bending moment is 93960, accordingly. In the case of a hollow cylinder whose wall thickness is the same as the thin portion of the outer walls of the hinge cells, $F = 122$ mm^2, $r_1 = 20$ mm, and $r_2 = 19$ mm. The bending moment is 23180. The actual hinge cell is therefore considerably more weakly constructed than the thickened portions of the wall adjoining on both sides. Simple observation of the dimensional relationships themselves makes this obvious. Indeed, the ratio of the bending moments is 4:1, i.e., the thickened portions of the walls of the hinge cells are approximately four times less flexible than the thin ones.

If the relationship between the structure and function of the tactile bristle presented above is correct, then it should make no difference to the resulting motor response whether the stimulation, namely the bending of the bristle, is caused by a solid object or by a fluid striking against it, for example, a jet of water. Contradicting this, Darwin (1876, pp. 263, 264) says that "drops of water or a thin, intermittent stream falling down from a certain height onto the filaments" produces no stimulation. Of course it was previously indicated by Goebel (1891, p. 202)., that raindrops usually run off the thin bristles without moving them. That will certainly be the case if the direction in which the drops are falling coincides with the lon-

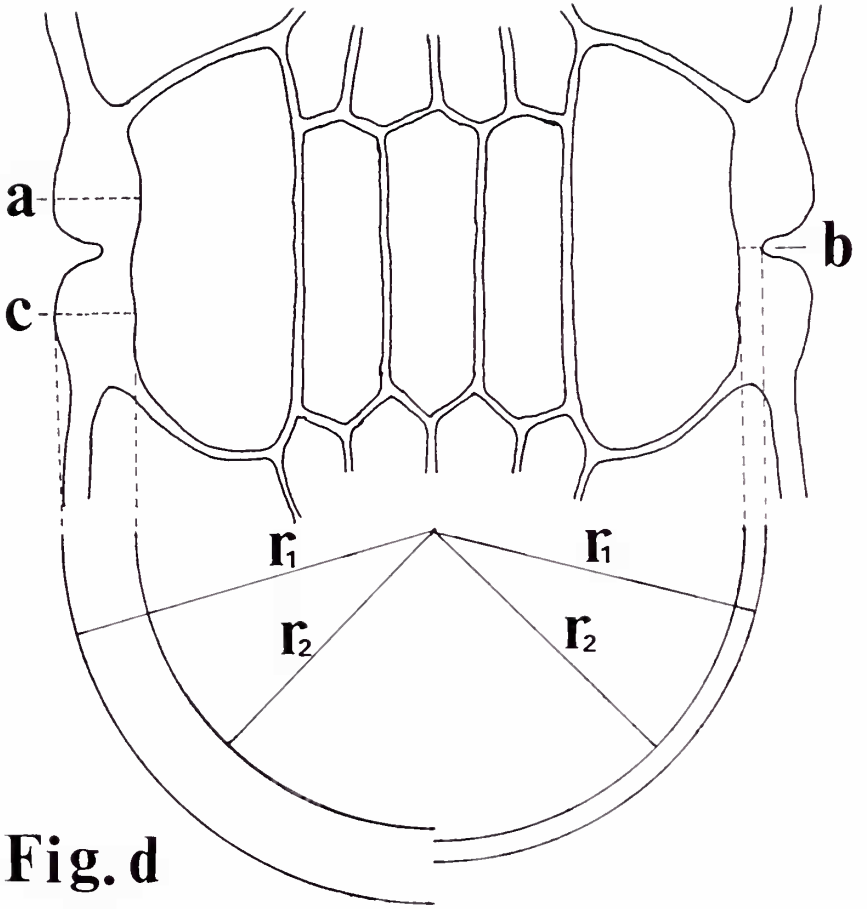

Fig. d

Fig. d. upper portion—a longitudinal section through the sensory cells of the trigger hair (c.f. Plate VI, Fig. 10). Lower right — a projection of the thickest part of the outer cuticle and cell wall illustrating one-fourth of the hollow cylinder used to compute the moment of inertia (I_c) of the thickest point. Lower left — a projection of the thinnest part of the outer cuticle and wall illustrating one-fourth of the hollow cylinder used to compute the moment of inertia (I_b) of the thinnest point. $I = F(r^2_1 + r^2_2)/4$ where F = the cross-sectional area of the projected cylinder, r_1 = the outside radius and r_2 = the inside radius.

gitudinal axis of the upright bristle, or forms an acute angle with it. If, however, a lateral jet of water is aimed at the bristles, then, as Balfour has shown, the motor response occurs immediately[14].

The tactile bristles of *Dionaea* must, according to the preceding material, be considered as very completely developed perception organs. If, on the whole, they are the most complete and most highly specialized organs of this species, then this presumes a very long period of evolution in the course of the phylogeny. This seems all the more likely since no other primitive bristles occur on the leaf which might have been the phylogenetic predecessor of the tactile bristles[15].

With regard to sensory physiology, it is highly interesting that sense perception is not exclusively limited to those highly specialized organs developed for that purpose. Various researchers, such as Darwin, Munk, Goebel and others agree that the upper side of the leaf blade is also sensitive to mechanical stimulation, albeit to a much lesser extent. Movement may also be caused by a wound.

A. Batalin 1877. Mechanik der Bewegung der insectenfressenden Pflanzen. Flora 35: 54-58; 105-111; 129-154.

M.A. Curtis 1834. Enumeration of the plants around Wilmington, N.C. Boston Journ. Nat. Hist. 1: pp. 123-127.

C. Darwin 1876. Insektenfressende Pflanzen trans. V. Carus (Stuttgart). p. 250.

Sydenham Edwards 1804. *Dionaea Muscipula*. Venus's Fly-Trap. Curtis Botanical Magazine. 20, plate 785.

J. Ellis. 1770. A botanical description of the *Dionaea muscipula* or Venus's flytrap, a newly discovered sensitive plant. Nova Acta Regae Societatis Scientarum Upsaliensis 1: 98-101.

K. Goebel. 1891. Pflanzenbiologische Schilderrungen (Marburg), pp. 57-72.

G. Haberlandt 1896. Physiologische Pflanzenanatomie. 1L (Aufl), pp. 481-482.

F. Kurtz 1876. Zur Anatomie des Blattes der *Dionaea muscipula*. in Reichter and DuBois-Reymond Arch. Anat. und Physiol. (Leipzig), pp. 1-29.

J. Lindley. 1848. An Introduction to Botany, 4th ed. (London).

J.M. Macfairlane. 1892. Contributions to the history of *Dionaea muscipula* Ellis. Contributions of the Bot. Lab. of the Univ. of Pennsylvania 1: 7-44.

F.J.F. Meyen. 1839. Neues System der Pflanzenphysiologie. 3: 545.

H. Munk. 1876. Die electrischen und Bewegungsterscheinungen am Blatte der *Dionaea muscipula*. in Reichter and DuBois-Reymond Arch. fur Anat. und Physiol. (Leipzig) pp. 30-203.

T. Nuttal. 1818. The Genera of North American Plants (Philadelphia), p. 277.

C.A. Oudmans. 1859. Over de prikkelbaarheid der bladen van *Dionaea muscipula* Ellis. Verslagen en Mededeelingen der k. Akademie van Wetenschappen 9: 320.

S. Schwendener. 1874. Das mechanische Prinzip im anatomishen Bau der Monokotylen (Leipzig), p. 23.

ENDNOTES

by Stephen E. Williams, Department of Biology
Lebanon Valley College, Annville, PA 17003

1. This very important experiment clearly shows that the "endpiece" or lever portion of the trigger hair which lies above the sensory cells of the "hinge" is not a part of the sensory system. Benolken and Jacobson (1970, J. Gen. Physiol. 56: 64-82), although they were unaware of Munk's experiments, did the same type of experiment — only in reverse. They sliced away the podium bit by bit and found that the electrical signals could be produced until they cut into the cell level of the hinge. Each of these experiments indicates that some or all of the cells at the hinge are sensory in nature, as Haberlandt states as the major thesis of this part of his work. However, it is also possible that other cells distal to the hinge cells have a sensory function as well, as has been pointed out by M. Williams and Mozingo (1971, Amer. J. Bot. 58: 538).

2. Haberlandt apparently is clouding the issue here. There is little doubt that the sensitive response is usually triggered by the hairs and that Munk and Darwin believed that to be so. The question is really whether the ability to respond is also in other parts of the leaf which, although sensitive, are more difficult to stimulate because of their structure or other factors or whether the hair is the only sensitive part of the leaf. They found other parts of the plant to be sensitive, a fact which is of some physiological interest and which Haberlandt recognizes in later parts of this chapter.

3. Zinc Chloride-iodine solution was a test for various polysaccarides which could be identified by the color they develop when treated with this solution.

4. Walls in this middle layer, which Haberlandt states are suberized, stain with Sudan black B in the same way that the walls of the endothermal layer in the *Drosera* tentacle stain (S. Williams, 1976, Proc. Amer. Phil. Soc. 120: 187-204). Lloyd (1942, *Carnivorous Plants*, p. 188) stated that he could "find very little if any suberization" in this layer. The methods used by both Lloyd and Haberlandt to detect suberization are not given. The Sudan stain, which dissolves in hydrophobic lipid structures such as cuticular wax, indicates that these walls are impermeable to water and are like the Casparian strips found in the walls of many glandular structures. There are important changes in the drawing

of this structure that were made in the second edition of *Sinnsorganne* (c.f. Fig. 10 and Fig. c).

5. The great significance of these structures is precisely due to the fact that they do not have a function. Like the leg bones of the python or our appendix they may be remnants of structures that had functions in ancestral organisms. Nearly all plant glands have an endodermis with Casparian strips of some sort. The presence of such a structure in the trigger hair of *Dionaea* is important evidence that this purely sensory structure may be derived from a secretory structure very much like the *Drosera* tentacle. The homology of the *Drosera* tentacle with the *Dionaea* and *Aldrovanda* trigger hairs is discussed in detail elsewhere (S. Williams, 1976, Proc. Am. Phil. Soc. 120: 187-204).

6. The correctness of Haberlandt's statement is easily confirmed by looking at the electron micrograph of the 1-2 μm thick cuticle of this region in Fig. 12 of M. Williams and Mozingo (1971, Amer. J. Bot. 58: 232-539).

7. Again M. Williams and Mozingo's (1971, Amer. J. Bot. 58: 232-539) observations agree with those of Haberlandt rather than those whose he is critical of. There are clearly no pores. However, the denticulations he reports are reported to be cuticular vesicles by M. Williams and Mozingo, who were observing sections of tissue less than 90 nm (0.09μm) thick in contrast to Haberlandt's 4μm thick sections. It is quite possible that denticulations which were attached to the cuticle have been cut through in a way that makes them look like vesicles in thin sections. The fact that they remain attached to the cuticle when Haberlandt hydrolized away the cellulose walls with sulfuric acid supports this interpretation (Plate VI, Fig. 11). Electron microscopic studies are best done in parallel with light microscopic work.

8. This mixture is also called Fleming's fixative. It has several variations containing different concentrations of chromic acid, osmic acid and acetic acid in water (Humason, 1967, *Animal Tissue Techniques*, p. 19).

9. These plasmodesmata were hypothesized to exist by Benolken and Jacobson (1970, J. Gen. Physiol. 56: 64-82) to explain the physiological data they observed. Haberlandt's drawings (Plate VI, Fig. 10 and Plate VII, Figs. 2, 3) clearly support their hypothesis but M. Williams and Mozingo (Amer. J. bot. 58: 538) report that these plasmodesmata do not exist. They do report large numbers of plasmodesmata connecting the sensory cells — an observation which Haberlandt failed to make. Because of the grat care with which he did his work Haberlandt's observations should not be taken lightly. However, M. Williams and Mozingo's (1971, Fig. 8) longitudinal sections show some plasmodesmata in thinner portions of the wall but not the large number reported by Haberlandt. It would be interesting to know just what Haberlandt was looking at.

10. I was unable to find Sudan black B positive walls in this part of the hair, even though it was in this region that I was expecting them. I started looking because of the analogy of these cells and the hinge cells with the sensory cells of *Aldrovanda*. If these granules are cutin they should take the Sudan stain. This observation should be repeated.

11. A thorough investigation of the distribution of plasmodesmata throughout the trigger hair and adjacent leaf blade is in order. This is the best way that the pathway over which action potentials spread can be determined. The pathway Haberlandt suggests here is questionable on the basis of a comparison of his description of the distribution of plasmodesmata with that of M. Williams and Mozingo (1971, Amer. J. Bot. 58: 532-539).

12. Haberlandt's emphasis on the cell membrane sounds very up to date. It is quite likely that the membrane is the site of reception rather than a number of other organelles mentioned in far more recent studies of the trigger hair.

13. The "bending moment" refers to the moment of inertia of the cross section of the tubular outer wall and cuticle of the hair (see Fig. d). The moment of inertia (I) together with a constant called Young's modulus (E) determines how much force it takes to bend the hair. One can determine a "stiffness factor" (S), where S = EI, that determines how difficult it is to bend any particular cross section through the hair. (See Thomas, *Calculus and Analytic Geometry*, Reading, Mass., pp. 550-551). Haberlandt is comparing the relative ease of bending the hair at two points along its length — that is, at the narrowest point of the hinge (point b) and at a nearby point (point a or c) where the wall and cuticle of the hair are very thick. A way to compare these is to find the ratio of the stiffness factors (S_c/S_b) at the two

points. Since $S_c/S_b = E_c I_c/E_b I_b$ it follows that $S_c/S_b = I_c/I_b$ if the Young's modulus (E) is the same at both points on the hair. The Young's modulus (E) depends on how easy the material in the wall and cuticle is to stretch. Haberlandt has assumed it is equally easy to stretch per unit cross-sectional area at both points and so he has computed $I_c/I_b = 4$. His conclusion is that it is four times as easy to bend the hair at the narrow place than at the broad places so that bending will tend to take place there. Haberlandt may have used a slide rule since his calculations are not exact, as may now quickly be ascertained with a calculator. The calculated values for I have far too many significant figures for the data anyway, so the precision is more than enough to justify the approximate 4:1 ratio of stiffness factors.

14. If the tendency of the trigger hair to require more stimuli for closure as the stimuli are delivered at less frequent intervals is added to this discussion, this would be a very accurate account of this phenomenon (c.f.s. Williams, 1980, CPN 9: 65, 75).

15. For a discussion of the evolution of this structure see S. Williams, Proc. Am. Phil. Soc. 120: 187-204.

Acknowledgments: Thanks are due to Dr. S. L. Jacobson and Dr. Y. Heslop-Harrison for reviewing the manuscript and to Mrs. Carla Lange for bibliographic help. Dr. James Scott of the Lebanon Valley College Foreign Language Department made major contributions to the translation.

Plate VII, Left Side

Dionaea muscipula

(Microtome sections)

Fig. 1. Part of a radial longitudinal section through the hinge of the tactile bristle; the sensory cell is pictured together with the protoplast (× 700).

Fig. 2. Cross section through the hinge of the tactile bristle; inside the ring of radial sensory cells is the central bundle, with cutin particles embedded in its cell walls. Fixed with alcohol, stained with paracarmine (× 660).

Fig. 3. Partition wall between two sensory cells; cross sectional view. The wall is penetrated by plasmodesmata; fixed with chrome-osmium acetic acid, stained with paracarmine (× 880).

Fig. 4. Part of a cross section through the hinge of a tactile bristle; the sensory cells touch at the upper ends, such that on the outside, the cross sections through the down-turned parts of the epidermal cells bordering above are still visible (× 660).

Fig. 5. Tangential longitudinal section (saggital section) through the sensory cells of the tactile bristle (× 700).

Fig. 6. Cross section through the upper part of the stimulator of the tactile bristle (× 480).

Plate VI, Right Side

Dionaea Muscipula

Fig. 10. Longitudinal section through the basal portion of the tactile bristle (× 440).

Fig. 11. Surface view of the hinge of a tactile bristle. The cuticle of the stimulus-sensitive cells is very finely denticulated on its inner side.

Drosophyllum lusitanicum

Fig. 14. The same, [surface view of two cells of the epidermal glandular layer] of a sessile gland.

Fig. 16. The same; [isolated protoplasts of the lateral glandular cells of a parietal tentacle, viewed from the side] the cells in question were situated more toward the apex (× approx. 900).

Fig. 17. Isolated proplasts of an apical glandular cell (× approx. 900).

Fig. 18 Surface view of some lateral glandular cells (× approx. 900).

Drosera longifolia

Fig. 19. Isolated protoplasts of two lateral glandular cells. After treatment with dilute sulfuric acid and staining with toluidine blue (× approx. 1000).

Drosera dichotoma

Fig. 21. Surface view of two lateral glandular cells of a cluster. Treated with Javelle water.

Fig. 22. Surface view of some apical glandular cells.

PLATE VII LEFT SIDE

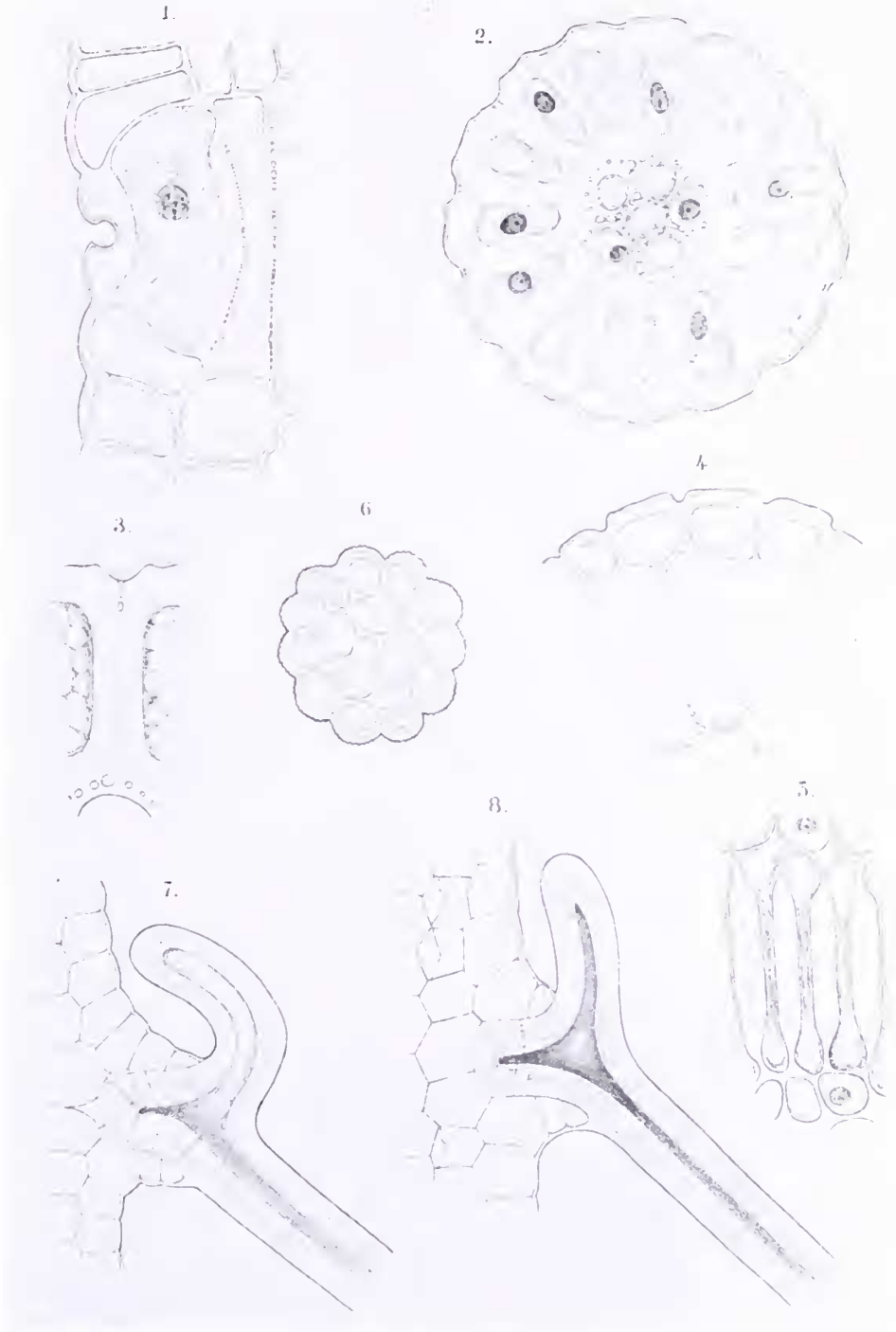

PLATE VI RIGHT SIDE